

## \*Tests of the Suitability of Hydnocarpus Oil for Injection

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In this department we have during the last few years received many requests for information regarding how to tell whether a sample of oil is good or not. We have also received many reports of trouble, pain and local reaction after the injection of hydnocarpus oil. We have therefore studied the matter closely, examining various specimens of oil physically and chemically, and correlating the findings with the results of therapeutic tests carried out in patients. This brief paper incorporates the results of this investigation and gives information which may be of use to people who prepare and distribute the oil and to doctors who wish to know how to tell good oil from bad.

Most, if not all, of the trouble with injections of hydnocarpus oil is due to the oil being badly prepared or badly stored or both.

### *The preparation of the oil.*

Hydnocarpus oil, if it is to be good, should be expressed from fresh ripe seeds. Bad fruits and seeds should be rejected. It is preferable that the outside of the seed should be removed before the oil is expressed. Most manufacturers, however, express the oil from the whole seeds. This introduces into the oil many particles of dust and vegetable matter which are adherent to the outside of the seeds. As will be explained later it is important that these particles should be removed as soon as possible by filtration.

### *Adulteration of oil.*

Adulteration of oil is not infrequently seen and may be the cause of trouble with injections. Oil sold by dealers of repute is usually unadulterated.

*Tests for adulteration:* The only real test for adulteration is to test specific rotation with the polarimeter. The exact method of doing this need not be described here. Anyone desiring to get samples of oil tested should send about 5 c.c. of oil to the Leprosy Department of the School of Tropical Medicine, Calcutta. The specific rotation of good hydnocarpus oil is usually

about  $+57^{\circ}$ . Oils giving a value less than  $+53^{\circ}$  are adulterated or decomposed oils which will probably give trouble on injection.

*Prevention of oxidation of oil.*

We have found that the irritant properties of oil are greatly increased by oxidation due to bad storage. The products of oxidation are irritant and consist chiefly of acids and other substances such as peroxides. All oil if kept for a long time undergoes a certain amount of oxidation and good oil stored even under good conditions for a year or two may become unsatisfactory for use. This indicates the necessity for using fresh oil but since the trees bear fruits only once a year, storage is necessary. There are certain factors connected with storage which greatly accelerate the process of oxidation. These are the presence in the oil of dirt, dust and water, and the exposure of the oil to air, heat and sunlight. Therefore oil should be perfectly free from water, should be filtered to remove all foreign particles, should be stored in air-tight containers which are absolutely full so that all air is excluded, and should be kept in a cool dark place. If these precautions are not taken oil may very rapidly deteriorate and cause serious trouble. Unfortunately manufacturers of oil not infrequently neglect all these precautions. For this reason it is advisable that all doctors who treat any number of leprosy patients should be able to carry out simple tests of oils supplied by dealers so that if the oil is bad it can be rejected at once. At the same time doctors should realize that the oil may be good on receipt from the dealer and may, because of improper storage, become bad later. Another important point which a doctor should know is that repeated sterilization of oil greatly aids oxidation and renders oil unsatisfactory for use. Oil should be sterilized once only and kept sterile.

*Tests for oxidation products in oil.*

The two chief products, the presence of which may be tested for, are acids and peroxides. Even good oil contains a certain amount of these but bad oil contains them in excess. The test for acidity is simple but does not give quite such a reliable indication of the irritating properties of oil as does the test for peroxides which is however more difficult.

(a) *Acidity.*—The acidity of oil is calculated in percentages of oleic acid. The percentage of acidity of really good oil is less than 1% but with an acidity of less than 3% oil

is usually suitable for injection. Between 3 and 5% it is very doubtful and over 5% it is usually bad.

A rough method of estimating the percentage of acidity of oil is here described:—

Take a 50 c.c. conical flask, pour in between 5 and 8 c.c. of pure ether and add 1 c.c. of oil carefully measured in a pipette, making sure that there is no oil adherent to the outside of the pipette. The oil remaining inside should be thoroughly washed out of the pipette by repeatedly sucking up and blowing out the ether. Shake the flask to dissolve the oil, add 15 c.c. of pure absolute alcohol, shake and then add a few drops of alcoholic solution of phenolphthalein indicator. Titrate the contents of the flask against  $\frac{N}{10}$  caustic soda<sup>1</sup> shaking the flask thoroughly and adding drop by drop from a burette until the pink colour appears and stays after shaking. Read the number of c.c. of  $\frac{N}{10}$  NaOH used and multiply by 3. This gives roughly the percentage of acidity if the above amounts are used.

(b) *Peroxides*.—Good oil contains only a small amount of peroxides but oxidized oil contains large amounts. The presence of peroxides can be detected chemically. The basis of the test is that peroxides have the power of liberating iodine from potassium iodide, and iodine in the presence of starch gives a marked colour reaction. The test is, however, applicable only to untreated oils as supplied by good dealers. Oils which have been extracted with a solvent or neutralized by caustic soda cannot be tested in this way. (See final paragraph).

The test may be performed either as a qualitative test or preferably as a quantitative test.

*Qualitative test.* The materials required are:—

(1) A solvent mixture consisting of chemically pure glacial acetic acid 2 volumes, and chemically pure chloroform 1 volume, (2) pure solid dry potassium iodide, (3) approximately 5% solution of potassium iodide prepared by dissolving potassium iodide in freshly boiled and cooled distilled water, (4) 1% solution of pure starch in distilled water, the solution to be boiled and cooled, (5) a strong thick walled test tube about 17 mm. in diameter and 20 cm. long fitted with a rubber cork.

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<sup>1</sup>(To be prepared by dilution of normal solution purchased from a reliable firm of chemists.)

The method is as follows:—

Take about 1 c.c. of oil in the test tube. Add about 1 gram of solid potassium iodide and about 5 c.c. of solvent mixture. Shake to dissolve the oil. The potassium iodide remains undissolved at the bottom of the tube. Boil vigorously on a flame for about 30 seconds. If marked excess of peroxide is present a brown colour at once develops. If there is no appreciable colour change, put the cork in the tube, cool under a tap and add 10 c.c. of 5% potassium iodide solution and shake. The contents of the tube are now a whitish emulsion. Add about 1 c.c. of the starch solution and observe any immediate colour change. The colour varies from a very light brown, almost pink, to a very dark indigo brown, according to the amount of peroxide present in the oil, the lighter shades of brown indicating small amounts and the darker shades large amounts. If the oil is suitable for injection only a light brown colour should be shown in the test.

*Quantitative test.* Materials required:—

In addition to the requirements for the qualitative test the following are needed:—

- (1)  $\frac{N}{500}$  Sodium thiosulphate solution prepared by dissolving 1 gram of crystals of chemically pure sodium thiosulphate and .05 gram of anhydrous sodium carbonate in 2 litres of distilled water freshly boiled and cooled. This solution does not keep well and should be freshly prepared from time to time with perfectly pure salts. For really accurate work it should be standardized by chemical methods, but for rough work this is not necessary.
- (2) A 250 c.c. conical flask with a glass stopper.

*Method.*—Weigh the test tube carefully. Weigh carefully 1 gram of oil in the tube then add approximately 1 gram of solid powdered potassium iodide. Run in about 19 c.c. of the solvent mixture washing down any potassium iodide which is adherent to the walls of the tube. Shake the tube to dissolve the oil. Heat the tube over a flame until it boils gently and then hold the tube into a vessel containing boiling water. The liquid rapidly boils and boiling is continued until the froth rises well up the tube, (about 20 to 30 seconds is usually necessary). At this point insert a rubber cork, shake vigorously for a few seconds and cool under a running water

tap. Measure about 20 c.c. of 5% potassium iodide solution and pour about 5 c.c. of it into the flask. Remove the cork from the tube and pour the liquid from the tube into the flask. Wash out the potassium iodide from the tube into the flask with the remainder of the 20 c.c. of potassium iodide solution.

Keep the stopper in the flask as much as possible. Pour into the flask about  $\frac{1}{2}$  c.c. of 1% starch solution; in the presence of peroxides a brown colour results. Titrate the contents of the flask against  $\frac{N}{500}$  sodium thiosulphate solution shaking thoroughly the whole time. The end point is reached when the brown colour goes and is replaced by the pale straw colour of the oil in emulsion. Read the number of c.c. of  $\frac{N}{500}$  sodium thiosulphate used. With a really good oil the reading should be less than 1 c.c. With readings less than 2 c.c. the oil is usually satisfactory. Higher readings indicate the presence or excess of peroxides.

#### *Purification of oxidized oil.*

If oil contains too much acid and peroxide it can be purified by washing one or more times with  $\frac{1}{2}$ % caustic soda and by passing steam through it, but this is a troublesome process. After neutralization the oil can be tested for acid as described above but the test for peroxide is no longer applicable.